EXPLORATION FOR ANTIMICROBIAL POTENTIALS OF SELECTED ETHNOMEDICINAL PLANTS

Research Protocol

For Registration for Ph.D. Programme

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May, 2021

JECRC UNIVERSITY, JAIPUR

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CERTIFICATE

This is to certify that this Research Protocol entitled **"EXPLORATION FOR ANTIMICROBIAL POTENTIALS OF SELECTED ETHNOMEDICINAL PLANTS "** by **Richa Thawani**, submitted in fulfilment of the requirement for registration in Ph.D. Program in **DEPARTMENT OF CHEMISTRY** under Faculty of Science of JECRC University, Jaipur, during the academic year 2021, is a bonafide record work carried out under our guidance and supervision.

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INTRODUCTION

Plants constitute the lowest part of the food chain. They produce their own food with the help of sunlight, carbon dioxide, chlorophyll and water. Our planet Earth, is named as “Green Planet” due to the presence of plants.

According to Foster and Anderson (1978), ethno medicine signifies the inclusiveness of health knowledge, values, beliefs, skills and practices of aboriginal people involving all the clinical and non- clinical activities that correlate to their health needs.

Rajasthan being the third largest state of India, inhabits about 205 medicinal plants (Tripathi *et., al* 1995)

Rajasthan consists of about 80% of rural population and nearly 50% of the tribal population belonging to different communities (Tripathi *et al.,* 1996). As tribal people are interrelated with forests so they acquire adequate knowledge of medicinal use of plants without knowing much about their scientific aspects.

Roots of *Epipremnum aureum* can absorb nicotine from soil which is further translocated via xylem to leaves where it get accumulated to a level observed more than which is present in nicotine rich *Nicotiana species* (Weidner *et al* .,2005)**.**

According to Yang and Liu (2011), *E.aureum* (*Pothos aurea*) is helpful for removal of indoor pollutants like xylene, benzene and formaldehyde.

Families like Apocyanaceae and Fabaceae possess many medicinal plants like *Abrus precatorius, Alstonia scholaris, Cassia occidentalis, Cassia sophera, Catharanthus roseus, Delonix regia, Plumeria acutifolia,Tamarindus indica, Vicia faba, Rauwolfia serpentina, Thevetia peruviana*,*Tamarindus indica, Vicia faba etc.* which have enormous utility in the treatment of malaria and related manifestations in Bangladesh (Bachar *et., al* ,2012).

There are many families of plant species like Zingiberaceae, Fabaceae, Solanaceae, Piperaceae, Malvaceae etc . in which ethno medicinal plants are found. There is a need to acknowledge and enlist those species of plants. There is abundant availaibility of medicinal plants,few among them are *Bauhunia variegata*, *Moringa oleifera, Oringanum mojorana, Cicer arietinum, Epipremnum aureum* etc. Their plant parts reveal many of the antioxidant and antimicrobial properties. Studies have been

performed to evaluate the phytochemical profiling, antimicrobial, antioxidant and anticancer activities of *Bauhunia variegata* (kachnar) leaf extracts (Mishra et. al, 2013).

Methanolic extract screening of leaves of *E. aureum* for antibacterial activity showed noticeable inhibitory action against Gram negative bacteria viz. *P. aeruginosa, E.coli, S.paratyphi A, K. pneumonia and S.typhi* (Mehta *et al*., 2013).

Roots of various medicinal plants contains various bioactive molecules which have the capability of curing many human diseases like cancer, diabetes, ulcer and liver diseases (Das *et al.*, 2014).

Various plant parts like bark, root, leaves, tree sap and gum are used for the treatment of numerous maladies like paralysis, heart disease, asthma, toothache, gas formation, indigestion, piles and many more .These plants belong to families like Liliaceae, Papaveraceae , Rubiaceae (Rahman and Keya ,*2015)*

A plant is made up of many different parts like roots, stem, leaves, flowers, fruits and seeds. These plant parts are sources of many powerful and potent drugs and possess their own medicinal properties (Shakya, 2016).

The use of medicinal plants for the treatment and prevention of many types of diseases is taking place for thousands of years. They possess numerous anti-oxidant, anti-inflammatory, anti-insecticidal, anti- parasitic, anti-hemolytic properties. These medicinal plants are used by rural and tribal people without having any knowledge about the scientific aspect and chemical composition of these plants (Bamola *et al.,*2018).

Moringa oliefera, member of family Moringaceae possess many of nutritional, antioxidant and phytochemical properties as almost every part of this plant including leaf, root, bark, seed, flower and pod has the components which are vital for the good health of livestock (Sonkar,2019). *Bauhinia variegata* is a medium sized deciduous tree which is abundantly used by tribal people for curing various diseases (Singh *et al*., 2019).

An annual herb *Spinacia oleracea* (Spinach), member of family Chenopodiaceae shows many therapeutic effects in the treatment of many ailments like diabetes, leprosy, asthma, urinary diseases, lung inflammation, joint pains, cold, fever and even diseases related to brain and heart (Miri and Roughani , 2019).

India possess a wide range of climate, topology and habitat which allows a wide range of vegetation to grow in India. Many varieties of plants are contributing to the source of medicines which increase the demand for medicinal plants (Rao and Ramakrishna**,** 2020)**.**

In the present investigations efforts will be made to enlist the Ethno medicinal plants with medicinal properties and screening of these plants for phytochemical properties. The purpose of screening is to justify and validate the use of ethno-medicinal plants by traditional healers for the treatment of various maladies.

AREA OF STUDY

Rajasthan, is the largest state of India and it is situated in the north western region of the country. Jaipur, also known as Pink city is located in the north eastern part of the state, is the capital of Rajasthan. Bikaner, Ajmer, Kota, Udaipur, Jodhpur are some of the major cities of Rajasthan. The capital city of the state is situated quite close to the capital city of the country, New Delhi, and the distance between the two cities is close to just 160 miles. The state of Rajasthan has borders with 5 states of the country, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, and Gujarat.

In present study the research will be focused for ethno medicinal plants from Pushkar in and around.

LITERATURE REVIEW

According to the latest Global Forest Resources Assessment (FRA) brought out by Food and Agriculture Organization (FAO) of the United Nations, India holds third position among those countries which have gained forest areas in the last decade.

An important aspect of research in Ayurveda is Drug research which is very important for maintaining and supplementing drugs and their calibration (Malaiya, 1997).

Tribal people in far off dense forests of the Aravalli hills of Mewar region are very much dependant on the natural remedies obtained from plants and this helps them to manufacture of traditional goods from them (Ketewa, 2003).

According to WHO more than 80% of people in the world depend on traditional medicinal system for their health care needs (Calixto, 2005).

Different parts of medicinal plants were used as medicine by the local traditional healers. Among the different plant parts, the leaves were most frequently used for the treatment of diseases followed by whole plant parts, fruit, stem, root, stem and root bark, seed, flower and latex (Muthu et al., 2006).

Plants are used in several systems of medicines like Unani, Ayurveda and Homeopathy (Khan, 2010). Herbal medicines are available for mild ailments like cuts and burns to major ailments like tumors and wounds in Indian and Chinese societies. It is quite obvious that herbal preparations are developed for an important event like child birth in the society. There is abundant knowledge among traditional people regarding the use of herbs for childbirth and affect fertility, pregnancy, parturition and postnatal period (Jain *et. al,* 2011).

Vegetative remedies' usage is on the leap in various advancing and advanced countries. Many disorders like urinary tract infections, vaginitis, gastrointestinal disorders, respiratory diseases, cutaneous infections, can be cured with the help of remedies prepared from some specific medicinal plants (Vaghasiya, 2012).

The traditional knowledge system in India is fast eroding. There is an urgent need to record all the ethnobotanical information among the diverse ethnic communities before the traditional culture is completely lost (Ram,2017).

Traditional knowledge on medicine means the use of plants available in the surroundings for the treatment of ailments (Sreejesh, 2018).

The forests are home for various communities of tribes who are fulfilling their needs from the plants available in these forests. While getting some useful plant extracts for healing their ailments, they gain a lot of knowledge about the remedial properties of the plants available in their surrounding but due to their scientific unawareness they do not get them properly documented (Sreejesh, 2018).

STATEMENT OF RESEARCH PROBLEM

"**Exploration for Antimicrobial Potentials of selected Ethno-medicinal Plants"**

OBJECTIVES OF STUDY

1. To conduct a survey of traditional healers of Pushkar in and around areas.
2. To enlist and process the selected plants with respect to their ethno medicinal properties
3. To analyse the phytochemical properties of the selected plants.
4. To perform the bioefficacy testing of the selected plant species.

METHODOLOGY

Instruments

Instruments like water bath, electronic weighing balance; distill water assembly, dryer/ oven, vacuum desiccators, sieve, centrifuge, shaker, thermostat, nutrient agar media, pre-autoclaved antibiotic preparing discs, conical flasks, incubator, forceps, autoclave, and inoculating loop, will be used in this study.

Collection

Survey of Tribal pockets of Pushkar in and around areas in Rajasthan will be carried out. Few interviews of the local healers of pushkar areas using medicinal plant will be done. Different plants with medicinal potential will be collected from these areas. More than 100 plants with different formulations will be screened out and tabulated. These plants will be mapped with different diseases. The resultant plants i.e *Bauhinia variegata* (kachnar) etc. with the potential therapeutic uses will be selected for the studies of bioefficacious properties.

Identification and processing of plant materials

All the samples will be authenticated by Herbarium, University of Rajasthan, Jaipur and processed. Selected plants will be further screened for:

Extraction – Reflux of the plant materials (250gms) with ethanol will be performed for 18 hours, then filtration will be done, the filtrate will be concentrated to dryness and will be utilised for further chromatographic studies. At the same time each plant material weighing about (100 g) will also be soxhlet extracted successively in petroleum ether/ dichloromethane ethyl acetate and alcohol/ methanol for 6 hours for

successive extraction process. The extract obtained will be filtered then evaporated to dryness and weighed. The dried extract of each plant material will be dissolved in 10 ml for performing TLC (Thin Layer Chromatography) and discs of 1 mg/ml, 5mg/ml and 10mg/ml concentration will be used for bioefficacy screening.

Bioactivities – Antimicrobial and Antioxidant – Various extracts, secondary metabolites rich fractions/ bioactives will be further screened for various bioactivities i.e. Antimicrobial – Antibacterial and Antifungal by disc diffusion method (Gould and Bowie, 1952), and Antioxidant assay (Takao *et al.,* 1994).

Antimicrobial, Antibacterial and Antifungal potentials of selected plants

During the previous decade the pharmacologists and herbalists were very much fascinated by the use of substitute medical therapy. Plants have always been a source of innovation for new and effective medicinal compounds which have proved to be a boon for human health and wellbeing. Herbal products are abundantly used all over the world. It has been revealed through many studies that plants possess many substances such as peptides, unsaturated long chain aldehyde, alkaloid constituents, some essential oils, phenols, and water, ethanol, chloroform, methanol and butanol soluble compounds .Due to the presence of these therapeutic beneficial compounds such plants have enormous implementation against human pathogens, including bacteria, fungi, or virus. Therefore, in the present research work plan, successive extractions of all the extracts will be performed and then screening of each extract will be done to attain bioactivity guided fractionation and finally will get to pure bioactives with antibiotic potentials (Gould and Bowie, 1952).

Antioxidant potential of selected plants

It has been investigated that free radicals generated by fast foods like pizza, burger, chowmein, momos etc. are fundamental cause of degenerative diseases. Free radicals can be trapped by the antioxidants. The dietary intake of antioxidant rich foods and the incidence of human disease are interrelated (Sies, 1993). It is observed that compounds are more active in their natural formulations than their isolated form (Khopde *et al.,* 2001).

Disc Diffusion Method

This method is used for detection of the antimicrobial activity of bacterial strains. It is based on the principle that compound soaked in paper discs will be diffused into agar media and will inhibit the growth of colonies of microbes resulting in the appearance of clear patches(Biemer, 1973).

EXPECTED OUTCOME OF THE STUDY

1. This study will be anticipated to provide recognition to the traditional healers for the treasure of knowledge about ethnomedicines which they are using in their tribal region.
2. This study will help in identifying the antimicrobial activity of selected ethno medicinal plants.

SCOPE OF THE STUDY

1. The study will help us to find out the antimicrobial properties of the selected ethno medicinal plants.
2. The present study will help in providing scientific recognition to the treasure of knowledge of traditional healers.

LIMITATIONS

The study takes in certain limitations-

1. Collection of ethno botanical plants will be focused mainly to regions around Jaipur.
2. Extraction will be done on petroleum ether(40-60°C) / Dichloromethane, ethyl acetate and methanol/ ethanol extract only

Year wise plan of work

1.	Literature review	Throughout the year
2.	Survey of traditional healers	0 to 4 months
3.	Collection and enlisting of Ethno-medicinal plants	4 – 9 months
4.	Experimentation for phytochemical and antimicrobial studies	9 – 25 months
5.	Statistical analysis	25 –29months
6.	Result and interpretation	29 – 31 months
7.	Preparation of thesis	31 – 36 months

Year wise plan

Activities/Months	0 –4	4 – 9	9 – 25	25 – 29	29 –31	31-36
Literature review						
Survey of traditional healers						
Collection and enlisting of plants						
Experimentation for phytochemical and antimicrobial studies						
Statistical analysis						
Result and interpretation						
Preparation of thesis						

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